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Potentiometric and Spectrophotometric Determination of the Dissociation Constants of Cefetamet

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Summary. The dissociation constants of cefetamet-Na have been determined using potentiometric titrations and spectrophotometry. The investigations were carried on in water solutions at constant temperature and ionic strength, and at different H_0 and pH values. Potentiometric investigations were performed at three different temperatures and ionic strengths. The concentration dissociation constants and the corresponding thermodynamic dissociation constants were calculated by a computer program. The mixed dissociation constants (pK') of cefetamet-Na have been determined spectrophotometrically in the H_0 range from −5.80 to 0.00 and at pH values from 0.00 to 12.70 and are in good agreement with values achieved by graphical methods as well as with those obtained by potentiometric methods. Based on the determined values, the thermodynamic parameters ($\Delta G, \Delta H, \Delta S$) were calculated at $I = 0.1$ M.

Keywords. Cefetamet-Na; Dissociation constants; Potentiometry; Spectrophotometry.

Potentiometrische und spektrophotometrische Bestimmung der Dissoziationskonstanten von Cefetamet

Zusammenfassung. Die Dissoziationskonstanten von Cefetamet-Na wurden mittels potentiometrischer Titrationen und auf spektrophotometrischem Weg bestimmt. Die Untersuchungen wurden in wäßriger Lösung bei konstanter Temperatur und Ionenstärke und verschiedenen H_0- und pH-Werten durchgeführt. Potentiometrische Messungen wurden bei drei verschiedenen Temperaturen und Ionenstärken vorgenommen. Die stöchiometrische Dissozation und die entsprechenden thermodynamischen Dissoziationskonstanten wurden mit Hilfe eines Computerprogramms berechnet. Die gemischten Dissoziationskonstanten (pK') wurden spektrophotometrisch im H_0-Bereich von −5.80 bis 0.00 und im pH-Bereich von 0.00 bis 12.70 bestimmt und stimmen sowohl mit Werten, die mit Hilfe der graphischen Methode erhalten wurden, als auch mit potentiometrisch bestimmten gut überein. Aus den ermittelten Werten der Dissoziationskonstanten wurden die thermodynamischen Parameter ($\Delta G, \Delta H, \Delta S$) für $I = 0.1$ M berechnet.

Introduction

Much interest has been shown in the chemistry of β-lactam antibiotics with respect to their biological activities. β-Lactam antibiotics, such as penicillins, cephalosporins, and oxacephalosporins, represent the most important class of drugs against infections and diseases caused by bacteria. The biologically active principle of these antibiotics is the β-lactam ring, the reactivity and selectivity of which towards biological substrates can be decisively modified by substituents. Cefetamet pivoxil (Fig. 1) is an orally absorbed prodrug ester of the microbiologically active cephalosporin, cefetamet. The prodrug ester is completely hydrolyzed to the active compound on its pass through the gut wall, the liver, or both [1]. Cefetamet is classified as a third generation cephalosporin which has excellent in vitro activity against the major respiratory pathogens and an enhanced stability against β-lactamases compared to penicillins and first and second generation cephalosporins [2].

Dissociation constants are among the most important physicochemical constants with respect to the absorption, distribution, and elimination of medicinal substances. Drug *pK* values can be used to predict possible precipitation in admixtures and solubilities in aqueous solutions as well as to provide optimum bioavailability by maintaining a certain ratio of ionized to unionized drug.

Having in mind the above arguments as well as the fact that we could not find any data on cefetamet dissociation constants in the available literature, it seemed to be of interest to determine its *pK* values, thus improving the understanding of its activity.

Results and Discussion

Potentiometric determination of the pK values of cefetamet-Na

The curves obtained by pH-metric titrations of cefetamet-Na with HCl solutions are presented in Fig. 2. Since the aqueous solution of cefetamet-Na showed *pH* values between 4.202 and 4.722 (at different temperature and ionic strength), it was necessary to perform the titrations in acidic solution in order to obtain both dissociation constants (K_1 and K_2). From these data, the formation function $\tilde{n}$ (i.e. the mean number of protons bound to the base (cef) could be determined at each point according to the Rossotti-Rossotti method [3] by means of Eq. (1) where *c*

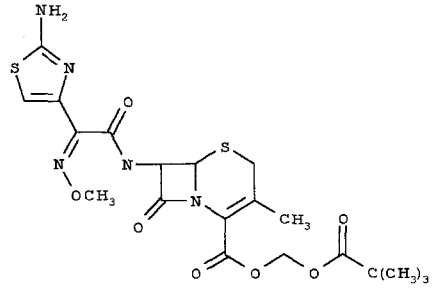


Fig. 1. Molecular structure of cefetamet-pivoxil

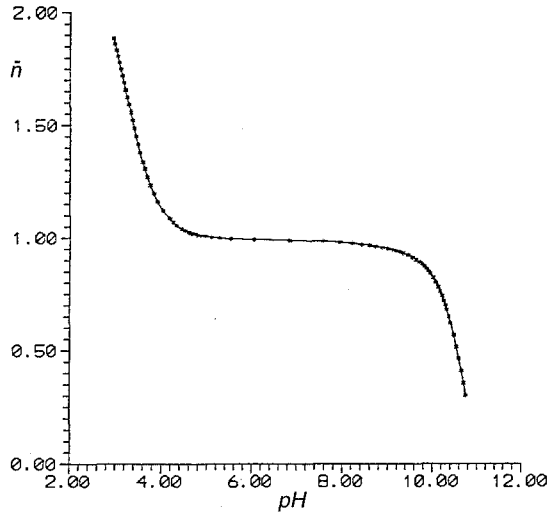


Fig. 2. Potentiometric titration curves of cefetamet-Na ($6.12 \times 10^{-3} M$) with $2.88 \times 10^{-2} M$ HCl (1) and $2.88 \times 10^{-3} M$ HCl (2)

denotes the stoichiometric concentrations and whereas square brackets indicate equilibrium concentrations corrected for the volume change during the titration. Eq. (1) allows to determine the experimental value for $\tilde{n}$ at each data point.

$$\tilde{n} = \frac{c_{cef} + c_{HCl} - [H_3O^+] + [OH^-] - c_{NaOH}}{c_{cef}} \tag{1}$$

The protonation curve ($\tilde{n}$ vs. pH) was obtained from the potentiometric data (Fig. 3). The maximum value for the formation function is about 2, indicating that two protons are available for acid-base reactions. The curve is composed of two distinct sigmoid steps, separated by a long plateau at $\tilde{n} = 1$ indicating that the values of pK_1 and pK_2 are separated by more than four units and, therefore, the two protonation steps can be treated independently.

In the pH range of 1–11, cefetamet undergoes an acid-base equilibrium as shown in Scheme 1.

The acidity constants of these processes are defined as

$$K_1 = \frac{[cef\,H^{\pm}][H_3O^+]}{[cef\,H_2^+]} \tag{2}$$

$$K_2 = \frac{[cef^-][H_3O^+]}{[cef\,H^{\pm}]} \tag{3}$$

In the pH range in which $cef H^{\pm}$ and cef^- are in equilibrium, $\tilde{n}$ is given by equation (4);

$$\tilde{n} = \frac{\sum_{i=1}^{N} i[cef\,H_i]}{\sum_{i=0}^{N}[cef H_i]} = \frac{[cef\,H^{\pm}]}{[cef\,H^{\pm}] + [cef^-]} \tag{4}$$

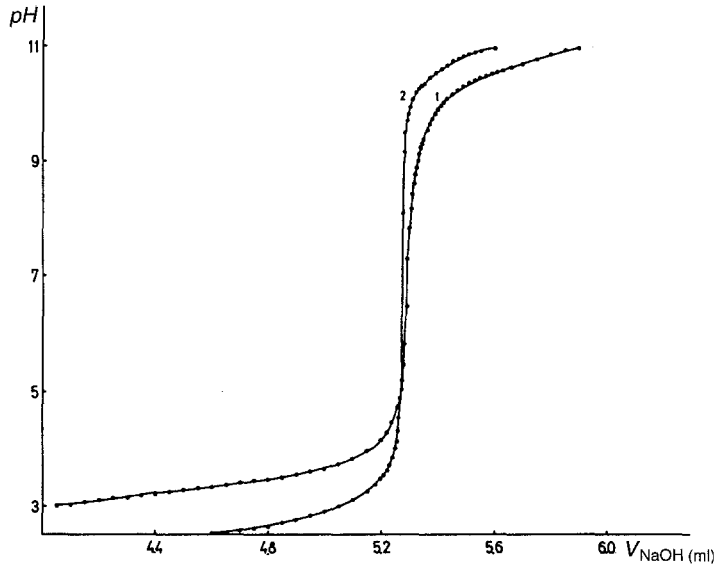
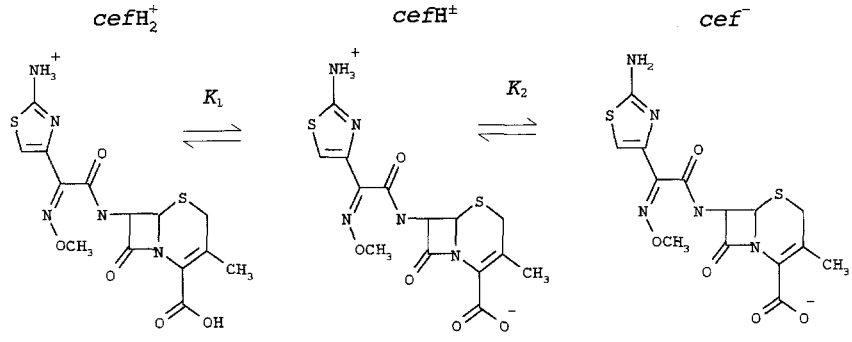


Fig. 3. Protonation curve of cefetamet-Na



Scheme 1. Protonation scheme of cefetamet

for the equilibrium of $cefH_2^+$ and $cefH^\pm$ species, the corresponding equation is

$$\tilde{n} = \frac{\sum_{i=1}^{N} i[cef\,H_i]}{\sum_{i=0}^{N}[cef\,H_i]} = 1 + \frac{[cef\,H_2^+]}{[cef\,H_2^+] + [cef\,H^\pm]} \tag{5}$$

The proton dissociation constants K_1 and K_2 were determined using the STECON-95a computer program. By combining Eqs. (2), (3), (4), and (5), the following linear expressions were obtained:

$$\frac{1-\tilde{n}}{\tilde{n}} = \frac{K_2}{[H_3O^+]}; \quad \frac{2-\tilde{n}}{\tilde{n}-1} = \frac{K_1}{[H_3O^+]} \tag{6}$$

Table 1. Concentration and thermodynamic constants of cefetamet-Na

$I(M)$	$T(K)$	pK_1	pK_2
0.20	298	3.08 ± 0.01	10.34 ± 0.01
0.10	295	3.06 ± 0.01	10.48 ± 0.01
0.10	298	3.07 ± 0.01	10.38 ± 0.01
0.10	303	3.02 ± 0.02	10.48 ± 0.02
0.05	298	3.08 ± 0.01	10.49 ± 0.01
0.00	298	3.09	10.56

The calculations were performed using 75 experimental points from each titration (six titrations were performed at three different temperatures and three different ionic strengths). The concentration and the corresponding thermodynamic dissociation constants are presented in Table 1.

Spectrophotometric determination of the pK values of cefetamet-Na

The absorption spectra of aqueous solutions of cefetamet in the UV region are changing with pH and H_0 (Hammett's function [4] for acidities below $pH = 0.0$) of the media (Fig. 4). In the range from $H_0 = -1.08$ to $pH = 1.70$, the spectrum of the aqueous solution of cefetamet shows two absorption maxima at 195 nm and 265 nm. According to the literature, the second absorption maximum for 3-cephems has been attributed to the interaction of the nitrogen lone-pair electrons with π electrons of the double bond [5] or to the sulfur atom, the ring nitrogen and

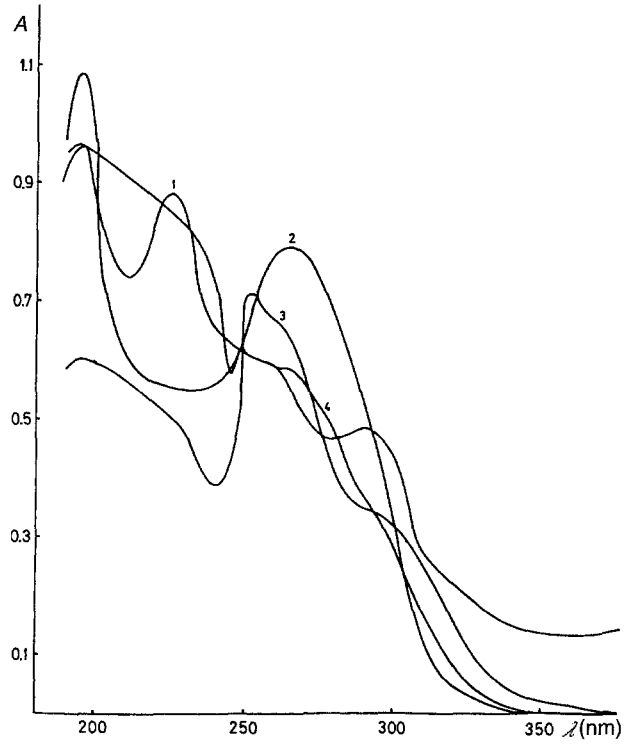


Fig. 4. Absorption spectra of cefetamet-Na ($5 \times 10^{-4} M$) in solutions of constant ionic strength ($I = 0.2$) and different pH and H_0 values; 1: $H_0 = -5.80$, 2: $pH = 1.70$, 3: $pH = 5.50$, 4: $pH = 12.70$

the double bond [6]. At higher H_0 values ($H_0 = -2.14$ to $H_0 = -5.80$), the maximum at 265 nm almost disappears, and a new one occurs at 225 nm, whereas two weakly pronounced absorption bands are recognizable at about 257 nm and 287 nm. The entirely new spectrum obtained in this strongly acid medium is probably due to the decomposition of cefetamet.

In the *pH* range of 1.70–5.50, there are evident variations in the absorption spectra. The second maximum is slightly shifted hypsochromically (251 nm), and an absorption band or shoulder occurs at about 300 nm. In the *pH* range of 5.50–10.0, there are no significant changes in the spectra, and in more alkaline medium, further variations can be noticed with one isosbestic point at about 290 nm.

On the basis of the absorption spectra recorded in H_2SO_4 and *Britton-Robinson* buffer solutions, the mixed dissociation constants pK_1' and pK_2' of cefetamet have been calculated according to Albert [7] using Eq. (7) where A_I and A_M represent the absorbance of the basic and the acidic form, respectively, of cefetamet, and A denotes the absorbance obtained at the given *pH* and wavelength.

$$pK' = pH + \log \frac{A_I - A}{A - A_M} \tag{7}$$

A graph of log $(A_I\text{-}A)/(A\text{-}A_M)$ *vs. pH* gives a straight line, the intercept at the abscissa indicating pK'. The pK' values can also be determined following the absorbance changes as a function of *pH* at wavelengths where these changes are most pronounced. The dependence of $\log(A - A_I)/(A_M - A)$ *vs. pH* at $\lambda = 275$ nm for pK_1', and at $\lambda = 280$ nm for pK_2' is shown in Fig. 5. The value of pK_1' is 3.10 and that of pK_2' is 10.60.

For the first constant, the calculation was performed at 275 nm and 282 nm, and for the second dissociation constant at 280 nm, 285 nm, and 320 nm. The results are shown in Table 2. The thermodynamic *pK* values are obtained from pK' constants using Eq. (8) [7].

$$pK = pK' + \frac{0.507 I^{1/2}}{1 + 1.5 I^{1/2}} \tag{8}$$

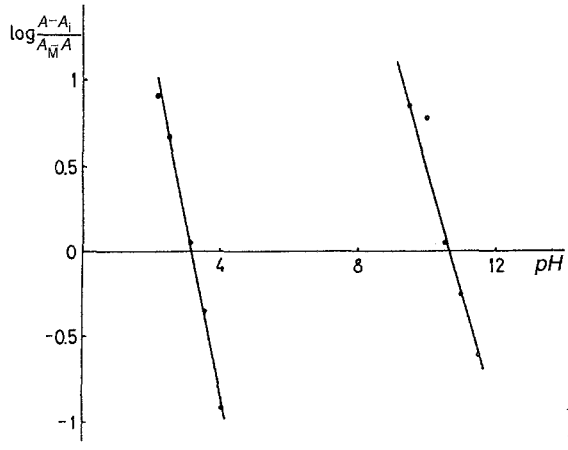


Fig. 5. Spectrophotometric determination of pK_1' at $\lambda = 275$nm, and pK_2' at $\lambda = 285$ nm; $I = 0.2$ *M* (KNO_3) $T = 298$ K

Table 2. Statistical data on the spectrophotometric determination of pK_1 and pK_2 ($I = 0.2M$, $T = 298\,K$); $\bar{x}$: mean value, $\bar{n}$; number of determinations, *SD*: standard deviation, *CV*: coefficient of variation, *pK*: thermodynamic acidity constant for $I = 0.0$

pK'	$\bar{x}$	n	SD	$CV(\%)$	pK
pK'_1	3.11	10	0.0207	0.67	3.25
pK'_2	10.60	12	0.0600	0.57	10.47

Table 3. Thermodynamic parameters for the dissociation of cefetamet ($I = 0.1\,M$, $T = 298K$)

Dissociation Step	Dissociation Constant	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (kJ/mol K)
pK_1	3.09	17.51	8.42	−0.031
pK_2	10.56	59.22	−9.96	−0.236
$pK_1 + pK_2$	13.65	76.73	−1.53	−0.267

The thermodynamic parameters $\Delta G, \Delta H$, and ΔS were determined at a constant ionic strength of $I = 0.1\,M$ and presented in Table 3.

According to the literature, all cephalosporins with a carboxylic group at position 4 have pK_1 values in the range from 1.5 to 3.4 [8, 9]. For older generation cephalosporins with an amino group in the side chain, pK_2 is about 7. However, third generation cephalosporins with the amino group bonded directly to the five-membered thiazolic ring show higher pK_2 values (e.g. ceftriaxone*, a compound structurally similar to cefetamet: $pK_1(COOH) \sim 3$, $pK_2(NH_2) \sim 10.8$ [10, 11]). This is in good agreement with the values obtained in our work. For an overview, see Table 4.

On the basis of the acidity constants determined, a distribution diagram of the percentage of mole fraction of each ionic species vs. *pH* was constructed (Fig. 6). It can be seen that in acid medium ($pH < 2$) cefetamet is protonated to give a cationic species $cefH_2^+$ which behaves as a diprotonic acid. Addition of base produces, in the first stage, the deprotonation of the carboxylic group ($pK_1 = 3.09$) yielding the zwitterionic form $cefH^{\pm}$ which is practically the only species existing

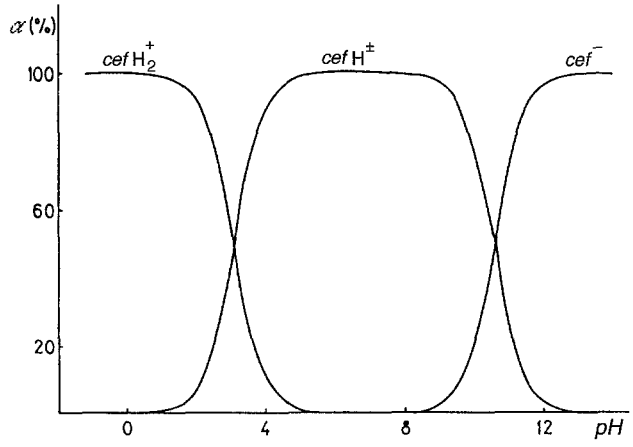
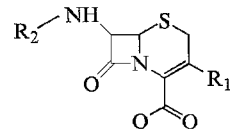
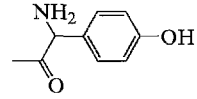
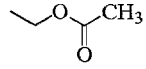
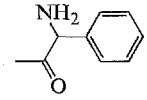
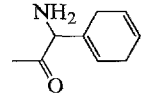
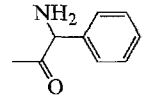
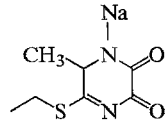
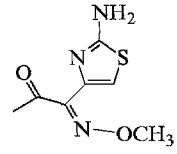
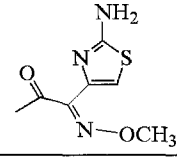


Fig. 6. Distribution diagram of cefetamet

Table 4. Chemical structures and dissociation constants of some cephalosporins



Cephalosporins	R_1	R_2	pK_1	pK_2
Cephalexin	$-CH_3$		2.56	7.24
Cephaloglycin			2.5	7.1
Cepharadine	$-CH_3$		2.63	7.27
Cefahlor	$-Cl$		1.5	7.17
Ceftriaxone*			~ 3	10.8
Cefetamet	$-CH_3$		3.09	10.56

in the *pH* range of 4.4–9.3 ($\alpha > 95\%$). In the second stage, when the *pH* is increased, the deprotonation of the amino group ($pK_2 = 10.56$) takes place, giving an anionic species cef^- which is predominant at > 11.8.

Materials and Methods

Apparatus

Potentiometric measurements were performed on a PHM-82 standard pH-meter (Radiometer Copenhagen) equipped with a combined electrode (GK2401) with an accuracy of ±0.001 *pH*. The response of the electrode was checked with standard Radiometer buffer solutions at $pH = 4.00$ and $pH = 7.00$. The potentiometric titrations were performed with a TTT-80 titrator with an autoburette ABU-12 (Radiometer Copenhagen) with accuracy of ±0.001ml. An U1 Ultra-Thermostat Medigen (Dresden) was used for maintaining a constant temperature (with an accuracy of ±0.1°C) during the titrations.

UV spectra in the wavelength range 190–450 nm were recorded on a Superscan 3 Varian UV/Vis spectrophotometer (quartz cell, 10 mm).

Reagents

All investigations were carried out with cefetamet-Na (standard) produced by Hoffmann La Roche (Basel, Switzerland). Other reagents were of analytical reagent grade: NaOH, $NaNO_3$, and HCl (Merck) for potentiometric investigations, and H_2SO_4, KNO_3, phosphoric, boric and acetic acid (Merck) for spectrophotometric investigations.

Solutions

For the potentiometric determination of dissociation constants, a solution of cefetamet-Na ($6.12 \times 10^{-3} M$) was used. Sodium hydroxide (0.1086 *M*, carbonate free [12]) was used for titrations. The concentration of sodium hydroxide was determined using a potassium hydrogen phthalate standard solution. Hydrochloric acid (0.8613 *M*) was used when titrations were performed in more acidic medium than the native cefetamet-Na water solution itself. A 2 *M* solution of $NaNO_3$ was used to keep the ionic strength constant.

For the spectrophotometric determination of *pK* values, a freshly prepared aqueous solution of cefetamet-Na ($5 \times 10^{-4} M$) was used. H_0 and *pH* in extremely acid solutions were adjusted using H_2SO_4 (in different concentrations) from $H_0 = -5.80$ to $pH = 1.70$. *Britton-Robinson* buffer solutions of double capacity were used for determinations in the *pH* range of 2.17–12.72. For that purpose, phosphoric, boric, and acetic acid (0.08 *M*) were stirred together with the corresponding volumes of sodium hydroxide solution (0.4 *M*). The ionic strength of 0.2 *M* was kept constant by addition of KNO_3 (2 *M*).

Potentiometric investigations

Titrations were carried out in a 20 ml jacketed glass vessel fitted with a stopper through which electrode, titrant delivery, and gas inlet tubes were inserted. An appropriate amount of cefetamet-Na was transferred into the glass vessel; the appropriate volumes of $NaNO_3$ (2 *M*) and HCl (0.8613 *M*) were added and diluted with doubly destilled water to 20 ml volume so that the final concentration of cefetamet-Na was $6.12 \times 10^{-3} M$. Before the start of each titration, a stream of nitrogen was passed through the solution, and the inert atmosphere was maintained during the titration. The measurements were carried out at three different temperatures (295 K, 298 K, and 303 K) and at three constant values of ionic strength (0.05, 0.1, and 0.2 *M*).

Spectrophotometric investigations

1.00 ml standard cefetamet solution was transferred to a 10 ml volumetric flask. 5.00 ml of *Britton-Robinson* buffer ($pH = 2.17$–12.72) and 0.80 ml KNO_3 (2 *M*) were added, and the flask was made up to the mark with distilled water. By the same procedure, cefetamet solutions of equal concentrations were prepared in H_2SO_4 (0.01 *M*–0.05 *M*) and in 0.1 *M* sodium hydroxide solution.

To prepare extremely acid solutions ($pH = 0.00$ and H_0 ranging from -0.28 to -5.80), 0.4 ml of 1.25×10^{-3} *M* cefetamet solution was transferred to a 10 ml volumetric flask, and 9.60 ml of H_2SO_4 (0.50 *M*–13.00 *M*) were added. The measurements were performed immediately after the preparation of samples against reference solutions at 298 K.

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